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Beyond the neuron - Cellular interactions early in Alzheimer's disease pathogenesis

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Abstract – The symptoms of Alzheimer's disease reflect a loss of neural circuit integrity in the brain, but neurons do not work in isolation. Emerging evidence suggests that the intricate balance of interactions between neurons, astrocytes, microglia, and vascular cells required for healthy brain function becomes perturbed during the disease, with early changes likely protecting neural circuits from damage, followed later by harmful effects when the balance cannot be restored. Moving beyond a neuronal focus to understand the complex cellular interactions in Alzheimer's disease and how these change throughout the course of the disease, may provide important insight into developing effective therapeutics.

[H1] Introduction

Alzheimer's disease (AD), the most common cause of dementia in the elderly, poses a growing problem for society as our population ages ¹. The overwhelming evidence that neuronal damage downstream of key pathological players causes cognitive decline has led to a "neuro-centric" view of AD pathogenesis. However, advances in our understanding of the molecular pathogenesis of AD approached from this neuro-centric perspective have thus far failed to translate into effective therapies. This reflects in part a failure to fully consider the effects of primary pathological changes on *all* cell types involved in neural system integrity, and the consequent perturbations of many key intercellular interactions.

In this review, we discuss the evidence supporting a role for interactions of multiple cell types in early AD pathogenesis, and how this can inform development of therapeutic strategies. Both the advances in fundamental understanding of disease and the outcomes of clinical trials over the past decade indicate the importance of cellular interactions early in the disease process ².

[H1] Early brain changes in AD

Alzheimer's disease is pathologically defined by the presence of amyloid beta (A β) accumulation in amyloid plaques, tau aggregation in neurofibrillary tangles, and brain atrophy caused by loss of neurons and synapses (Box 1). In addition to these definitive pathological lesions, the brain undergoes an innate immune response including large changes in the phenotypes of microglia and astrocytes. A substantial amount of evidence supports the amyloid cascade hypothesis of disease pathogenesis, which states that alterations in A β initiate the disease and set off a chain of events including accumulation of toxic forms of tau that cause downstream neuron death ^{3,4}. This is strongly supported both by the genetics of familial AD

implicating A β production and aggregation in causing disease and by the pathological accumulation of A β deposits beginning many years before symptom onset. Along with the changes in A β early in disease, there is an early loss of synapses which tracks with disease progression. There are also early changes in non-neuronal cell types including an inflammatory response of glia around plaques and disruptions to vascular function including reduced cerebral blood flow and blood-brain-barrier disruptions (table 1).

[H2] Risk factors point to multiple cells

The interest in cellular interactions in AD pathogenesis is increasing as both genetic and epidemiologic evidence point to a causative role for changes in the innate immune system, metabolism, and vasculature. These have initiated a boom of research in the field to understand the non-neuronal contributors and cell-cell interactions in initiating changes in the brain in the early stages of disease ^{2,5-8}. Early genetic evidence implicated the apolipoprotein E epsilon 4 allele (APOE4) as a potent risk factor for AD ⁹. APOE is highly expressed in astrocytes and disease-associated microglia in the brain, implicating a role for these cell types in pathogenesis. However, it was not until the more recent genome-wide association (GWAS), whole-genome and whole-exome sequencing studies implicating multiple AD risk genes expressed predominantly in non-neuronal cells, that the interest in non-neuronal contributors to AD has really taken hold (table 2). In particular, genetic evidence has directly linked microglial function to AD. Several genes involved in the innate immune system and inflammation increase AD risk, including triggering receptor expressed on myeloid cells 2 (TREM2), complement receptor 1 (CR1), CD33, and INPP5D. Astrocytic metabolism gene variants, in addition to APOE4, have also been implicated in AD pathogenesis including clusterin (CLU, also known as apolipoprotein J) and sortilin-related receptor 1 (SORL1). Interestingly, several genes that are highly expressed in oligodendrocytes or oligodendrocyte precursor cells (OPCs) have also been implicated in AD risk, although the role of these cells in the disease is much less studied than that of neurons, microglia and astrocytes.

Non-genetic risk factors also point to an important role of interactions of multiple cell types in disease pathogenesis. The mantra “what is good for your heart is good for your brain” is central advice for dementia prevention based on a large amount of evidence implicating vascular risk factors such as high blood pressure, obesity, type 2 diabetes, and a sedentary lifestyle, in substantially increasing AD risk ⁶. More recently, direct interactions of AD pathological proteins with the vasculature, have suggested changes in vascular function and stability ^{10,11}. All of these risk factors implicate non-neuronal cell types including vascular cells and glia in disease. While the mechanisms of neurodegeneration initiated by interactions between cell types and the timing of these interactions remains unclear, many recently discovered pathways converge on synapse degeneration as will be discussed later.

[H1] Inflammation and the immune response

The brain’s inflammatory response to toxic stimuli involves multiple cell types and cellular interactions. Both microglia and astrocytes respond to toxic stimuli in the brain by altering their gene expression, morphology, and secretomes, which have cascading effects on other cell types, including neurons. The dramatic envelopment of amyloid plaques by glial cells, and the evidence supporting inefficient A β clearance as a pathogenic pathway in sporadic AD have driven much of the work surrounding inflammation to focus on the role of glia in clearing A β . Clearing toxic forms of A β from the brain should be neuroprotective, however, A β also has effects on glial function, which can render them neurotoxic.

[H2] Role of glia in amyloid clearance

Microglia accumulate around plaques in AD and are well known for their ability to phagocytose A β . This begins at the early stages of disease as plaques accumulate in the neocortex. Many mouse models of this early stage of disease exist, which express familial AD-associated mutations in APP and PS1 and develop plaques and associated gliosis in the absence of neurofibrillary tau pathology or overt neuron loss. In one

such model, microglia form a barrier around plaques which prevent accumulation of toxic oligomeric A β “hotspots”¹². However, chronic microglial activation leads to a more damaging proinflammatory state¹³. For example, activated microglia are thought to recruit and activate other microglia through a self-perpetuating loop of cytokine release and receptor expression¹⁴. Ablating CX3CL1-positive microglia in plaque-bearing mice results in plaque growth, but no change in number, in support of an important role for these cells in limiting plaque expansion¹⁵. However, this finding is inconsistent as previous work in the same APP/PS1 model showed that a 90% pharmacological reduction of CD11b-positive microglia had no effect on plaque formation or maintenance¹⁶. Furthermore, ablating CSF1R-positive microglia in 5xfAD mice has no effect on amyloid burden, but does improve spine loss, neuron death and cognition^{17,18}. Soluble A β is thought to be much more neurotoxic than plaque fibrils, and recent evidence also supports a role for microglia in clearing soluble A β by secreting A β -degrading enzymes in a process regulated by complement receptor 3¹⁹. In contrast to the data showing microglial ablation results in plaque growth, other data indicate that blocking proliferation of microglia may be beneficial to cognition in mice^{17,18}. Thus, despite a clear role for microglia in clearing A β , it is not yet clear whether microglial activation is protective or harmful, or more likely, whether this depends on the stage of the disease. Further, in many of the studies examining microglia in AD models discussed here, it has not been possible to distinguish between microglia, brain macrophages and infiltrating bone marrow-derived cells. More detailed investigations will be needed to determine whether the effects observed are due to microglia or other myeloid cells.

Advances in single-cell profiling technology are starting to unravel some of these microglial mysteries both by helping to differentiate the different myeloid cell types and to tease out how these cells change upon activation. Recent data indicate that there are likely not just on/off states but a continuum of glial activation²⁰⁻²³. Single-cell RNA sequencing (RNAseq) in an AD mouse model revealed disease associated microglia (or DAMs), which accumulate around plaques and exhibit reduced expression of homeostatic genes and increased expression of genes involved in phagocytosis²³. Interestingly, two of the upregulated genes in DAMs are genetic risk factors for AD, APOE, and TREM2, providing a link between these molecules and microglial activation that may explain some of the risk these polymorphisms confer. More detail on the link between TREM2 and APOE recently emerged in a transcriptomic analysis of microglia showing that TREM2 can be activated by APOE signalling in disease-associated microglia²⁴. Interestingly, TREM2 binds to ApoE and clusterin (another genetic risk factor) and facilitates microglial uptake of A β , which is impaired by AD associated variants, at least in cell culture²⁵. A more complex disease-stage dependent role of TREM2 is becoming evident from *in vivo* studies. In one model of AD, TREM2 knockout was found to ameliorate amyloid pathology early in disease but exacerbate pathology later in the disease process²⁶, highlighting the variability in effects of microglial phenotypes at different stages of disease. While the data are not yet all in agreement, several studies suggest a protective role in which upregulation of TREM2 causes microglia to phagocytose A β . In support of this idea, genetic reduction of TREM2 levels in mice and the R47H TREM2 mutation in both mice and humans, reduced microglial or monocyte association with plaques²⁶⁻³⁰, implying that reductions in TREM2 reduce the “barrier” of microglia around plaques that may protect surrounding tissue³¹. Lack of TREM2 appears to reduce the ability of microglia to sense their environment and respond appropriately, resulting in reduced phagocytosis³².

Reactive astrocytes accumulate around plaques and are thought to play a role in A β degradation, thus appearing to act in a neuroprotective manner³³. This effect may be due to the increased expression of insulin degrading enzyme (IDE), which is observed *in vitro* in cultured astrocytes and *in vivo* in astrocytes near amyloid plaques³⁴. Alternatively, astrocytic matrix metalloproteinases (MMPs) may be involved in extracellular A β breakdown. Numerous MMPs are expressed in astrocytes and their levels increase following A β exposure *in vitro*³⁵ and in the brains of AD mice³⁶. Conversely, astrocytes may contribute to peri-plaque pathology by releasing pathological factors such as S100 β . This protein is enriched in AD brain and levels positively correlate with the number of dystrophic neurites within plaques³⁷. APOE is also

expressed in astrocytes and is thought to play a role in astrocytic amyloid clearance (as well as initial deposition and remodelling of A β into dense core plaques). Expressing human APOE isoforms indicates that APOE4 exacerbates plaque deposition in mouse models of AD compared to APOE2 or APOE3³⁸. In contrast, complete ablation of APOE in mice reduces plaque deposition^{39,40}. These APOE null data remain incompletely understood, but may have more to do with amyloid production and seeding in neurons than with amyloid clearance by astrocytes. A β also induces Ca²⁺ dysregulation in astrocytes *in vitro*^{41,42} and *in vivo*⁴³, and astrocytes from human post-mortem AD brain show alterations in expression of genes associated with Ca²⁺ signalling, contributing to impaired astrocyte function.

[H2] Glia and tau pathology

Tau pathology correlates more strongly with neuron loss and cognitive decline in AD than amyloid pathology, and extensive tau pathology throughout the brain is only present at later stages of the disease process downstream of initial insults. However, tau pathology does accumulate in the entorhinal region early in the disease and the spread of tau out of the medial temporal lobe is thought to be important for moving from the early stages of the disease in which brain circuits compensate well for pathology, to the later stages of progressive worsening of symptoms⁴⁴. There is some evidence that non-neuronal cell types may play a role in tau induced neuron death and perhaps also in its spread through neural circuits. As well as their well-known plaque associations, glia accumulate in the vicinity of neurofibrillary tangles and the burden of glia positively correlates with tangle burden in human AD brain⁴⁵. Further, gliosis occurs in non-AD tauopathies and tau pathology can accumulate in astrocytes⁴⁶. In support of interactions of glia with tau-induced neurodegeneration, transplantation of human neural precursor cells into a mouse model of tauopathy resulted in glial cell differentiation that protected mice from neuron loss⁴⁷. Both APOE and TREM2 have also recently been linked to inflammation in tau models. TREM2 deficiency in a mouse model of tauopathy reduced inflammation and prevented neurodegeneration at late stages of disease⁴⁶, however TREM2 reduction in a different tauopathy model examined at an earlier stage showed an exacerbation of tau phosphorylation⁴⁸. Furthermore, overexpression of TREM2 in microglia of P301S mice (a model of tauopathy), reduced tau phosphorylation and suppressed secretion of pro-inflammatory cytokines⁴⁹. These emerging data on TREM2 indicate that it may have different effects on tau pathology at different stages of disease (similar to the emerging story with A β), or at least that it has different effects in different mouse models. Although the data examining the relationship between TREM2 and tau are still sparse, they do urge caution in pursuing therapeutic strategies to activate TREM2 before we fully understand the role of this molecule in the disease.

Similarly, most work examining effects of APOE4 on AD have focused on A β or synapse loss, but there are now hints that APOE may also affect tau pathology, likely through modulating glial function. In a mouse model overexpressing frontotemporal dementia associated P301S mutant tau, knocking out APOE protected mice from neurodegeneration, while knocking in human APOE exacerbated neurodegeneration in an isoform specific manner with APOE4 being most toxic⁵⁰. Further, profiling of gene expression showed an increase in pro-inflammatory and a decrease in homeostatic gene expression in microglia and an increase in inflammatory profiles in astrocytes⁵⁰. It remains to be seen which cell types are important in mediating these APOE isoform dependent effects on neurodegeneration, as APOE is largely produced in astrocytes and microglia but its expression is also induced in other cells in the CNS.

Microglia may contribute to tau spreading through the brain. One recent study reported that after tau virus injection into the entorhinal cortex, ablation of microglia reduced phosphorylated tau, reduced tau spread from entorhinal cortex to dentate gyrus, and lowered proinflammatory cytokine levels⁵¹. Other studies have increased microglial activity by blocking or genetically removing the fractalkine receptor, which is constitutively activated by neuronally-released fractalkine (CX3CL1) to suppress microglial activation. Genetic removal of CX3CL1 results in increased tau phosphorylation in wild type and hTau overexpressing mice, via microglia-derived soluble factors⁵². In support of this, CX3CL1 overexpression in

the rTg4510 model, suppressed microglial activation and subsequent tau pathology⁵³. While interesting, there are caveats to these studies including small animal numbers and the possibility that reducing microgliosis likely reduced levels of inflammatory cytokines which in themselves could contribute to the spread of tauopathy. Further work is necessary to understand the potential role of microglia and also astrocytes in the early spread of tau through neural circuits.

[H2] Glial phenotypes change during ageing

In addition to their effects on amyloid and tau pathology, glial changes may be important for other aspects of AD pathogenesis independent of the amyloid cascade. Age is the largest risk factor for AD, yet we do not fully understand why age renders the brain susceptible to neurodegeneration. A recent genome-wide analysis of microglia in mice showed distinct region-dependent transcriptional identities that changed with age⁵⁴. Similarly, analysis of astrocytes from different regions of mouse brain during ageing showed age-associated and region specific transcriptional changes⁵⁵. Interestingly these age-associated astrocyte changes were reduced in mice lacking microglial secreted cytokines, indicating important interactions between microglia and astrocytes during ageing⁵⁵. In support of the translational relevance of these age-associated changes observed in mice, we observe differences in the distribution of microglia and astrocytes in different brain regions in healthy aged human brain⁵⁶, suggesting that regional differences in ageing of glia could contribute to selective vulnerability in age-related neurodegenerative diseases like AD. In a recent mouse study profiling the transcriptome of microglia, ageing, APP/PS1 overexpression, and P301L tau overexpression all induced a similar network of translational changes strongly implicating APOE in driving a network of changes resulting in production of the cytokines CCL3 and CCL4⁵⁷. The effects in this study were more pronounced in microglia from female mice. While this is a single paper utilising AD animal models, it points to the intriguing idea that the AD risk factors of age, APOE isoform, and female sex may converge on similar inflammatory pathways that are induced by the pathological proteins that accumulate in the disease.

[H2] Glial effects on neuron death

Neurons begin to die early in AD pathogenesis. By the time symptoms are detectable, estimates indicate that over half of neurons in layer II of the entorhinal cortex are lost⁵⁸. Microglial phenotypes and their effects on neuron death have recently been shown to change with disease stage. In a mouse model of neurodegeneration downstream of p25 overexpression, microglia appear to proliferate early, then later initiate a harmful host of innate immune responses⁵⁹. Microglia also influence neuron death in a human cell tri-culture system in which neurons and astrocytes expressing mutant APP are cultured in a three-dimensional chamber and microglia are added in a surrounding, connected chamber at later time points⁶⁰. In this system, infiltrating microglia contribute to neuron death in a pathway involving Toll Like Receptor 4 and interferon- γ . This has several limitations including overexpression of mutant APP and the use of an immortalized microglial cell line, but it does support a role for microglia in inducing death of human neurons^{60,61}. While these results from model systems are intriguing, it will be important to examine other models and human brain to fully understand how microglia change with disease stage and which changes may contribute to neurodegeneration. It will also be important moving forward to distinguish microglia from infiltrating microglia-like macrophages which enter the brain during disease and may have distinct roles compared to resident microglia⁶²⁻⁶⁴.

Astrocytes are also able to modulate neuron death via their expression of APOE. In mice, APOE3 expression in neurons or astrocytes and APOE4 expression only in astrocytes protected APOE null mice from excitotoxicity, while neuronal expression of ApoE4 was not protective and led to neuron death after excitotoxic challenge⁶⁵. While excitotoxicity has not been established as the cause of cell death in AD, this study provides proof of principle that the cellular source of ApoE4 influences neuronal susceptibility to neurodegeneration, and indicates that glia may impact cell death independently of amyloid.

[H2] Cellular cross-talk in inflammation

Along with their independent roles in neuroinflammation, there is increasing evidence for interactions between microglia and astrocytes in destructive feed-forward loops in AD. Work from Ben Barres' group showed that secretion of cytokines by microglia can activate astrocytes causing them to lose some of their physiological functions and become toxic to neurons⁶⁶. In AD models, two studies showing that IL-10 worsens amyloid-related phenotypes in mice also implicate microglial IL-10 in boosting astrocytic expression of APOE, which then looped back to decrease microglial appetite for A β ^{67,68}. The complement system also plays a role in microglia-astrocyte cross talk. In a recent study examining both plaque bearing transgenic mice and cultured astrocytes and microglia, A β was found to activate astrocyte expression of NF- κ B, which caused extracellular release of complement 3. This in turn caused microglial and neuronal C3a receptor activation, which impaired cognitive function and altered microglial A β phagocytosis⁶⁹. Immune system-related genes including clusterin and complement C3 are differentially expressed in laser-captured astrocytes from human AD brain compared to control brains⁷⁰, further implicating an inflammatory immune response in multiple cell types in AD.

Microglia and astrocytes both secrete cytokines in the AD brain. Treating cultured microglia with amyloid induces secretion of numerous proinflammatory mediators such as IL-1 β , IL-6 and TNF- α , as well as reactive oxygen species⁷¹. All of these factors can be released in differing combinations depending upon the pathological context and have significant detrimental effects on the surrounding milieu. Importantly, some of these molecules can induce the activity of γ -secretase, leading to enhanced A β production⁷². Exposure of cultured astrocytes to oligomeric A β similarly induces astrocyte activation⁷³ and to the release of proinflammatory cytokines such as IL-1 β and TNF- α and the gaseous synaptic modulator, nitric oxide (NO)⁷⁴. APOE genotype influences the inflammatory response of glia to A β exposure. APOE4 expression in a mouse model of AD with plaque deposition resulted in increased microglial and astrocytic reactivity and increased release of inflammatory cytokines, particularly in plaque-associated microglia⁷⁵. TNF- α has received a lot of attention as TNF receptor-1-knockout mice have significantly lower levels of amyloid production and deposition in an AD mouse model⁷⁶. In mouse models of familial AD that develop plaques and in wild-type animals injected with oligomeric A β , A β is associated with increasing levels of cytokines, confirming that A β induces cytokine release in vivo^{77,78}. While much work focuses on pro-inflammatory cytokines, there is also evidence that the anti-inflammatory cytokine IL-10 worsens amyloid pathology and cognition in mouse models^{67,68}. Understanding the complex role of cytokines will be important, particularly since cytokines may contribute to neuron death⁷⁹.

Complex cellular responses to systemic infection may also contribute to disease pathogenesis. The controversial idea that pathogens contribute to the initiation of AD has been around for decades^{80,81}, but has received a recent boost from 2 papers examining herpes infection in human AD and in a mouse model of disease. An extensive analysis of multiple human cohorts found higher levels of herpesvirus 6 and 7 in brain tissue from AD cases than controls, and computational analyses indicate that the viruses may regulate known Alzheimer's genes⁸². The increase of virus in the brains of people with AD could plausibly be due to the known disruption of the blood brain barrier in disease, which allows these very common viruses to get into the brain. More mechanistically convincing is the recent study, which showed that herpes simplex virus 1 infection leads quickly to amyloid deposition in an AD mouse model and a human stem cell derived neuronal culture model⁸³. This builds on work indicating that A β has antimicrobial properties⁸⁴. Whether this is important to AD pathogenesis remains controversial, particularly since plaque deposition is not sufficient to initiate full-blown Alzheimer's disease⁴⁴. However, it is possible that these processes may turn out to play a causative role in disease initiation, and there is no doubt that interactions of multiple cell types from the vasculature through glia and neurons are involved in this type of response to infection.

[H2] White matter and oligodendrocytes

While there is substantial evidence supporting a role for microglia and astrocytes in AD pathogenesis, there has to date been less investigation of a potential role for oligodendrocytes or their precursor cells (OPCs). White matter changes, as observed by MRI scans, are frequent in AD with recent studies showing associations of white matter damage with both amyloid⁸⁵ and tau pathology⁸⁶. Intriguingly, neuropathological studies do not generally reflect substantial white matter changes, although post mortem evidence of volume changes would be difficult to observe.

Early on, Braak observed that the pattern of neurofibrillary tangle deposition in AD parallels the developmental pattern of myelination⁸⁷, indicating that neurons with late-myelinating axons may be more vulnerable to degeneration, although the molecular link between these observations remains uncertain. Some gene variants that increase AD risk are predominantly expressed in oligodendrocytes or their precursor cells (table 1), and emerging evidence suggests that myelination is involved in neuronal plasticity⁸⁸, indicating that changes in these cells may contribute to the disease. Incubation of cultured oligodendrocytes with amyloid peptides, leads to apoptotic cell death⁸⁹ and injection of amyloid into the rat corpus callosum leads to axon damage, oligodendrocyte death and reactive gliosis around the injury⁹⁰. Inflammatory conditions such as those observed in AD can hinder the remyelination process, and prevent OPCs from differentiating to oligodendrocytes⁹¹. Although not yet fully substantiated, these potential oligodendrocyte processes provide further support for the importance of interactions between cell types in disease.

[H1] Cellular interactions in synapse loss

For almost three decades it has been known that the strongest neuropathological correlate with cognitive decline in AD is synapse loss⁹². Synapse loss occurs early in the disease and continues to parallel cognitive decline throughout disease progression⁹²⁻⁹⁶. The exact mechanisms driving this loss are yet to be fully elucidated, although it is evident that both amyloid and tau exert harmful effects on synapse integrity⁹⁷. Strong focus has been placed on the neuronal components of the synapse as excitatory glutamatergic synapses have historically been considered dipartite structures, consisting of a presynaptic terminal and postsynaptic spine. However, detailed imaging studies have revealed that many exist as tri- or even quadripartite structures with glial processes found in close proximity to the synapse. These non-neuronal synaptic components play important roles in synapse development, maturation and disease. Prior to synapse loss in AD, amyloid and tau accumulate in the synapse leading to disrupted function of glutamate receptors and synaptic kinases, altered calcium dynamics and spine disassembly⁹⁸. These direct effects on the neuronal components of the synapse are well established and supported by a growing literature describing presynaptic, postsynaptic and trans-synaptic effects of these pathological factors⁹⁷. However, the disease-driven disruption of glial function and their subsequent influence on synaptic physiology remain an area of intense research.

[H2] Immune cascades and synapse loss in AD

Over the past few years, data have emerged implicating both astrocytes and microglia in activity-dependent synaptic pruning during development⁹⁹⁻¹⁰³. The classical complement cascade has emerged as a key part of this developmental synaptic pruning¹⁰⁴⁻¹⁰⁶. In the developing mouse brain, synapses expressing high levels of complement components C3 and C1q are actively engulfed and phagocytosed by complement receptor 3 (CR3) positive microglia^{100,104}. Recent data indicate interactions between astrocytes and microglia in developmental synaptic elimination as microglial secretion of inflammatory molecules including C1q induces astrocyte activation, which causes neurotoxicity, at least in cultured cells⁶⁶. Conversely, astrocytes can recruit microglia to eliminate synapses. By releasing transforming growth factor β (TGF- β), astrocytes in the retina increase the expression of complement protein C1q on nearby synapses thus priming them for complement-dependent phagocytosis by recruited microglial cells¹⁰⁷. Intriguingly, the astrocytic elimination of synapses was also recently shown to be modified by APOE isoform. In young mice, ApoE2 increased astrocytic phagocytosis of synaptic terminals, whereas ApoE4 decreased astrocytic phagocytosis¹⁰⁸. Complement C1q was also low in APOE2 expressing mice and high in APOE4 mice, suggesting that ApoE4

may prevent astrocytes performing physiological C1q-dependent synapse clearance, leading to a build-up of senescent synapses ¹⁰⁸.

While much of the evidence for the involvement of the complement cascade in synapse elimination comes from studies of developing retinal ganglion cells in rodents, this cascade may also be involved in synapse degeneration in AD. Several genes associated with the complement system have emerged as AD genetic susceptibility factors, including complement receptor 1 (CR1) and clusterin ¹⁰⁹. Furthermore, many components of the complement cascade are significantly up-regulated in the AD brain ¹¹⁰. Evidence from mouse models suggests that amyloid-induced complement activation leads to microglial phagocytosis of cortical synapses ^{111,112}. In mice, soluble amyloid drives the up-regulation in C1q levels before the deposition of amyloid in plaques and, C1q knock-out significantly reduced the synaptotoxic effect of amyloid ¹¹¹. Genetic removal of complement C3 from APP/PS1 mice (APP/PS1;C3-KO) rescued synapse loss in the hippocampus, and knocking out CR3 (the receptor for C3) resulted in less synaptic engulfment by microglia following amyloid challenge ¹¹¹. APP/PS1;C3-KO mice have revealed some clues as to the roles of glia in amyloid clearance. In 16 month old APP/PS1;C3-KO mice, plaque burden was increased compared to APP/PS1, however they showed an altered glial response to amyloid plaques, namely decreased glial infiltration of plaques and lower levels of proinflammatory cytokines such as TNF- α and IFN- γ ¹¹². This associated with partial preservation of synapse density in the hippocampus and improved cognitive flexibility, suggesting that C3-mediated synapse loss is a feature in aged and AD brain ¹¹². The idea that microglia are phagocytosing synapses in the human AD brain remains controversial. Even in rodent slice culture, there is some debate about whether microglia actively eat postsynaptic dendritic spines with one study proposing a role for microglia in synaptic circuit remodelling by “nibbling” presynaptic terminals and facilitating postsynaptic filopodia formation ¹¹³. On balance the human genetic data and mouse model data strongly support a role for complement and microglia in synapse degeneration, but the details of this remain to be determined, particularly what is happening in human AD brain.

Immunological systems and cytokines beyond the complement system also contribute to synapse degeneration in AD. For example, the major histocompatibility complex 1 (MHC1) immune pathway has been shown to influence synapse function and formation ¹¹⁴. MHC1 associates with PSD-95 and can interact with a number of immunoreceptors, including paired immunoglobulin-like receptor B (PirB) ¹¹⁴. Neuronal PirB associates with synapses and plays a critical role in ocular dominance plasticity (ODP). When PirB is knocked out, ODP is enhanced ¹¹⁵ however in APP/PS1 models of AD, defective ODP is a very early marker of synaptic dysfunction ¹¹⁶. It has since been discovered that PirB acts as a receptor for amyloid oligomers and this interaction induces synaptic dysfunction and synapse loss by disrupting the synaptic cytoskeleton ¹¹⁷. Removal of PirB could rescue synaptic plasticity deficits and behavioural changes in AD mice ¹¹⁷. Importantly, amyloid oligomers have a nanomolar affinity for the human orthologue of PirB, leukocyte immunoglobulin-like receptor B2 (LilrB2) and although LilrB2 levels are not altered in AD brain, downstream signalling pathways are significantly upregulated ¹¹⁷. This suggests that in human AD brain, amyloid activates LilrB2 and increases its downstream signalling, leading to altered neuronal actin organisation and ultimately synapse loss. Further support of a microglial role in A β -mediated synapse loss comes from a study of the APP/PS1 mouse model with knockout of TDP-43 in microglia. TDP-43-KO increased microglial phagocytic activity and led to enhanced amyloid clearance and concomitant exacerbated synapse loss ¹¹⁸. This provides an intriguing link between the pathological proteins involved in AD and Amyotrophic Lateral Sclerosis (ALS), which also exhibits synapse loss associated with cognitive decline ¹¹⁹.

[H2] Excitotoxicity

In addition to the potential involvement in innate immune cascades, astrocytic end-feet at synapses are likely to play a role in synaptic dysfunction and loss in early AD, through modulation of excitotoxicity. Adenosine 2A receptors (A2ARs) are expressed on astrocytes and respond to synaptic adenosine by inducing a number of cellular changes, including a reduction in glutamate uptake ¹²⁰. Astrocytic A2ARs have a negative

role in learning and memory processes in mouse models of AD and high levels of these receptors are found on human astrocytes in AD ¹²¹. Therefore, excitotoxicity in AD may be driven by increased astrocytic A2AR expression on astrocytes, leading to decreased glutamate clearance and excessive synaptic activity. Alternatively, genetically lowering glutamate transporter-1 (GLT-1) expression in AD mice, results in an earlier onset of cognitive deficits ¹²² and amyloid is known to increase the lifetime of synaptic glutamate by inducing the internalisation of GLT-1 from astrocytic membranes ¹²³. Furthermore, restoring GLT-1 expression in an AD mouse model prevented synaptic pathology and cognitive decline ¹²⁴. Therefore, amyloid-induced alterations in GLT-1 location and function may increase synaptic and extrasynaptic glutamate, thus driving excitotoxicity in AD. In support of this, it is believed the beneficial role of Memantine in patients with moderate-to-severe dementia may be due to the blockade of extrasynaptic NMDA receptors, which are activated by excess glutamate ¹²⁵.

[H2] Risk genes influence synapse loss

Astrocytes are also likely to influence synapse dysfunction and loss due to their production of ApoE and its effects on synaptic A β ¹²⁶. We demonstrated a role for ApoE4 protein in targeting oligomeric A β to synapses, where it is associated with synapse degeneration in human AD brain ¹²⁷. In mouse models, it is clear that the synaptic effects of ApoE4 are not cell-autonomous, as we observed that bathing the cortex in ApoE4 expressed by viral infection of ependymal cells worsens synaptic phenotypes ³⁸. ApoE modulates A β metabolism and clearance ^{128,129}, and ApoE4 increases the oligomerization of A β ¹³⁰, which is toxic to synapses ^{131,132}. Interestingly, the ApoE4 effect is not just a feature of neurodegenerative disease but also plays a role in synaptic integrity in non-pathological cognitive ageing. ApoE plays a role in dendritic spine maintenance ¹³³, presynaptic terminal composition and glutamate synthesis ¹³⁴ and synaptic plasticity ¹³⁵, with ApoE- ϵ 4 isoforms having significant detrimental effects in these studies. A recent study utilising transgenic mice that express human ApoE isoforms, discovered an isoform-specific change in the phagocytic properties of astrocytes ¹⁰⁸. Non-demented individuals from the Lothian Birth Cohort in their 9th decade of life exhibit a faster rate of cognitive decline if APOE4 positive, compared to those with APOE2 or APOE3 alleles ¹³⁶. Future work on the well-characterized Lothian Birth Cohort subjects will help to clarify the role of factors such as APOE in cognitive ageing through deep phenotyping of genetic, psychosocial, and neuropathological factors ^{56,137,138}.

In addition to the clear links between A β , glia, and synapse loss, there are indications that tau mediated synapse loss also involves glia. Early studies in the P301S mouse model of tauopathy revealed that microglial activation coincided with the onset of synapse loss ¹³⁹. This occurred before neuron loss and tau tangle formation, placing active microglia at the right place and time to induce synaptic alteration ¹³⁹. The effects of TREM2 modulation of tau-induced synapse degeneration have not yet been fully explored, but one study showed that overexpressing TREM2 in microglia in these mice improves cognition and prevents loss of synaptic proteins ⁴⁹. There are also hints that astrocytes modulate synapse degeneration in tau mice. Cultured astrocytes from the P301S tauopathy model exhibit functional deficiencies that result in a failure to support neurons in culture ¹⁴⁰. GLT-1 is decreased in P301L mice indicating that astrocytic clearance of glutamate is impaired by tau expression in this model ¹⁴¹, which may contribute to excitotoxicity. Extracellular tau oligomers also disrupt astrocytic Ca²⁺ signalling, reduce astrocytic ATP release, and impair neuronal synaptic function in culture ¹⁴².

Together, these data strongly support the notion that multiple cell types contribute to synaptic changes (figure 1). This opens promising avenues to prevent or reverse synapse degeneration and potentially to prevent the spread of pathological proteins through neural circuits.

Fig 1

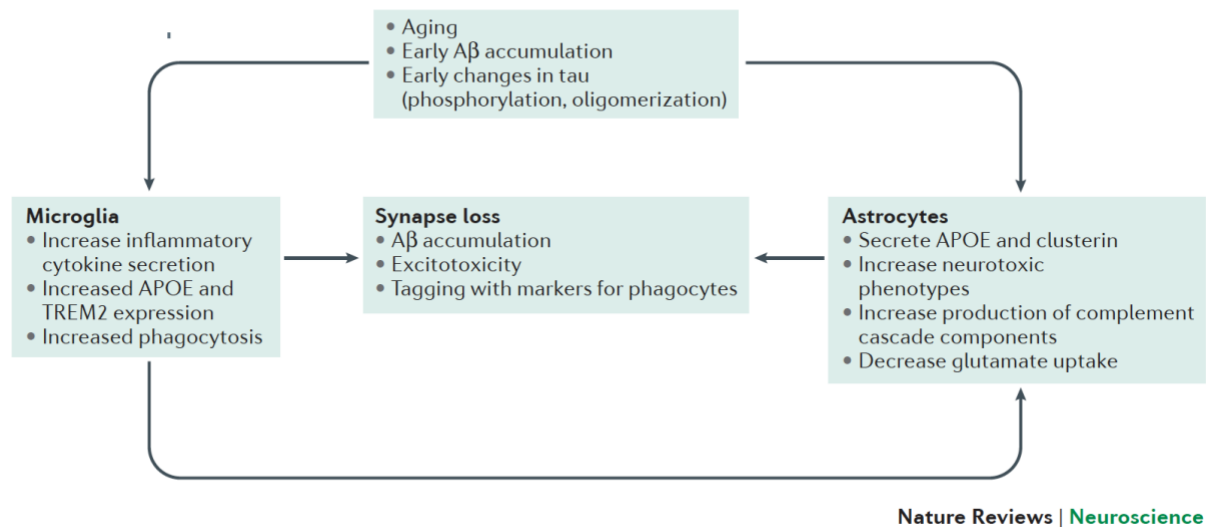


Figure 1: Synapse loss occurs early in AD pathogenesis. Emerging data indicate that microglia, astrocytes, and cross-talk between these are involved in synapse degeneration. Ageing, early pathological changes in A β and tau, and chronic hypoperfusion due to vascular impairments all contribute to synapse loss directly and indirectly via effects on microglia and astrocytes. Microglia and astrocytes also have direct effects on synapse degeneration and there is cross-talk between the glial pathways impacting synapses.

[H1] Role of the NVU in neurodegeneration

The brain is an energy hungry organ, dependent upon blood vessels for delivering oxygen and nutrients and for waste removal. An intricate control system has evolved to both protect the brain from components in the blood that are toxic to brain cells (the blood-brain barrier, BBB) and to regulate blood flow to different brain regions with fluctuating energy demands. This Neurovascular Unit (NVU) contains almost every cell type found in the brain, all working together in a tightly regulated manner. Vascular cells (endothelial cells, pericytes, and vascular smooth muscle cells) form the walls of the vessels. These are surrounded by astrocyte end feet, the terminal processes of astrocytes. Perivascular microglia and macrophages survey the influx of blood-borne molecules into the brain¹⁴³. The different cell types in the NVU communicate and are interdependent. For example, endothelial cells can promote proliferation and differentiation of both neural precursors and oligodendrocyte precursor cells^{144,145}, and via the release of trophic factors, also protect neurons from injury¹⁴⁶.

Strong epidemiologic evidence shows associations between vascular risk factors and risk for AD, and further that mid-life statin use to control high blood pressure is protective against AD¹⁴⁷. Recent studies show reductions in incidence of AD in the UK¹⁴⁸, and while the causes for this remain unknown, scientists speculate that better access to healthcare and lowered cardiovascular risk factors may be contributing to reduced incidence. Disruptions in NVU certainly cause vascular dementia⁷ and likely also contribute to the early stages of AD pathogenesis. It has been proposed that damage to the NVU starts a cascade involving reduced cerebral blood flow and BBB disruption, which when followed by a “second hit” of A β pathology leads to Alzheimer’s disease⁸. In animal models, vascular changes have been observed very early in the disease process. Reductions in cerebral blood flow and vascular reactivity are the first changes observed in a model overexpressing Swedish mutant APP^{149,150} and APP/PS1 mice exhibit ultrastructural changes in microvasculature before cognitive impairment is observed¹⁵¹. Tau overexpressing mice also develop vascular abnormalities, even those expressing wild-type human tau, which do not develop overt neurodegenerative phenotypes¹⁵². In humans, using dynamic contrast enhanced magnetic resonance imaging (MRI), decreases in hippocampal vascular volume have been observed in mild cognitive impairment (MCI) subjects¹⁵³, and increased BBB permeability has been observed even at an early stage of

AD¹⁵⁴. Brain glucose uptake measured by [¹⁸F]fluoro-2-deoxyglucose PET imaging is impaired in AD patients very early in the disease process¹⁵⁵. While these imaging studies have relatively small sample sizes (6-15 subjects per group), they do point to a link between vascular damage and AD. Large biomarker studies also point to the role of vascular malfunction as an early event in AD. In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, a data-driven model spanning 30 years of disease progression suggests that vascular dysregulation is one of the earliest events in Alzheimer's disease¹⁵⁶.

Along with direct effects of oligodendrocytes on myelin integrity discussed earlier, vascular changes can also impact white matter. White matter hyperintensities detected by T2-weighted MRI have emerged as an important biomarker of white matter damage. While the pathological causes and consequences of these hyperintensities remain to be fully determined, they have been associated with small vessel disease, glial cell death, microglial and endothelial cell activation, demyelination, axonal loss, and tau pathology in overlying cortex, linking many aspects of the NVU^{86,157,158}. A well-powered imaging study from the Dominantly inherited Alzheimer Network (DIAN) found white matter hyperintensities in people with familial AD mutations that began 6-22 years before symptom onset¹⁵⁹.

There are some hints at mechanisms linking vascular changes to AD, with the strongest evidence favouring defective clearance of A β from the brain to the periphery and chronic hypoperfusion as two of the contributors to sporadic AD. The routes of clearance of A β from the interstitial fluid, and indeed the routes of brain extracellular fluid drainage in general is not entirely clear and has been hotly debated over the past decades¹⁶⁰. While A β can be degraded in the parenchyma, a large part of A β clearance from the brain appears to be via the vasculature drainage, driven at least in part by para-arterial influx of cerebrospinal fluid (CSF) that causes convective fluid flux forcing interstitial fluid (ISF) out through para-venous efflux (known as the glymphatic system)^{10,161-164}. Meningeal lymphatics have also been recently implicated in A β clearance in rodents¹⁶⁵. In this study, impairment of lymphatic drainage impacted glymphatic exchange of CSF and ISF¹⁶⁵, implying that lymphatic and glymphatic drainage processes may be functionally linked – perhaps via effects on glia. Intriguingly, both production and clearance of A β change with the sleep-wake cycle with higher levels of A β in CSF and ISF reported during periods of more brain activity in mice and humans^{166,167}. This is doubtless due in part to the increased secretion of A β with increasing neuronal activity¹⁶⁸, but astrocytes are most likely involved in this process as well since glymphatic clearance is enhanced during sleep¹⁶⁹. Several genetic risk factors for AD including APOE, clusterin, and PICALM have been implicated in vascular clearance of A β ¹⁷⁰⁻¹⁷². Polymorphisms in aquaporin-4 are associated with modulation of cognitive decline in AD¹⁷³, which could be important because of the role of aquaporin-4 in the influx of CSF into the brain as part of the glymphatic system¹⁶⁴. Similarly, inducing pericyte loss in APP overexpressing mice by knocking down pericyte-deficient platelet-derived growth factor receptor- β resulted in exacerbated amyloid pathology. Pericyte loss also caused accumulation of tau pathology and neurodegeneration¹⁷⁴. However, the role of pericytes in regulating cerebral blood flow and protein clearance from the brain remains controversial¹⁷⁵.

The role of the NVU in AD goes beyond protein clearance. Hypoperfusion, hypoxia, and breakdown of the BBB are all thought to contribute to pathogenesis, processes which all involve complex interactions of multiple cell types⁸. For example, in mouse models of early AD, hypertension enhances A β induced NVU dysfunction and promotes BACE activity and subsequent A β production¹⁷⁶. Tau pathology has also recently been linked to changes in brain microvasculature. Mice overexpressing human wild-type tau or FTD associated mutant tau both develop changes to blood vessel structure which are associated with obstructed blood flow¹¹. These data indicate that changes in neuronal expression of tau impact endothelial cell biology, further supporting an important role for cell-cell interactions in AD.

As observed in parenchymal inflammation, perivascular microglia likely play a protective role early in the disease process by phagocytosing A β and potentially also phagocytosing extracellular tau as it propagates

from cell to cell. In support of this idea, in Tg2576 mice, decreased microglial perivascular accumulation correlated with increased deposition of cerebral amyloid angiopathy (CAA) and decreased survival of remaining microglia, which developed a pro-inflammatory phenotype and impaired the BBB¹⁷⁷.

Hypoperfusion due to vascular changes and neurotoxic changes including glial activation both lead oxidative stress through generation of reactive oxygen species (ROS), which can contribute to a negative spiral of degeneration. Accumulation of ROS can result from excessive production due to increased activity of superoxide-producing enzymes such as NADPH oxidase, or inhibited function of anti-oxidant enzymes such as superoxide dismutase (SOD)¹⁷⁸. This fine balance of pro- and anti-oxidant activity is particularly vulnerable in the brain due to its high abundance of polyunsaturated fatty acids, high metabolic rate and relatively low expression of antioxidant molecules¹⁷⁹. However, given the devastation observed in the human AD brain it is no surprise that oxidative stress is evident, the important question is whether oxidative stress is a cause of AD pathogenesis, or is a result of it. Animal models suggest that oxidative stress occurs before or in tandem with amyloid plaque formation^{180,181} and human studies suggest that oxidative stress represents an early pathological event in Alzheimer's disease¹⁸². Oxidative stress can result in neuronal death; however ROS can also have detrimental effects on other cell types in the brain.

Cerebral amyloid angiopathy (CAA), the build-up of amyloid on cerebral vasculature, can lead to vascular oxidative stress, via the generation of ROS, and this exacerbates vascular dysfunction. Animal models suggest that NADPH oxidase is the major source of toxic free radicals, as genetic removal of Nox2 (a catalytic subunit of NADPH oxidase) counteracts amyloid-induced, ROS-dependent, vascular dysfunction¹⁸³. Furthermore, vascular endothelial cells and perivascular microglia/macrophages express the amyloid-binding receptor CD36¹⁸⁴. This receptor increases CAA and is important in the amyloid-induced oxidative stress within the neurovascular unit¹⁸⁴. Interestingly, genetic removal of this receptor results in reduced CAA, reduced vascular dysfunction and improved cognitive function in Tg2576 mice, despite no change in cortical amyloid plaque burden¹⁸⁴. More recent animal work has suggested the source of amyloid-dependent oxidative stress is CD36-positive perivascular macrophages¹⁸⁵. Depleting perivascular macrophages in Tg2576 mice with clodronate, led to decreased amyloid-dependent vascular dysfunction and this was dependent on CD36 and Nox2 expression¹⁸⁵. This confluence of reactive oxygen species, loss of bioenergetics, and inflammatory responses involving the neurovascular junction, as well as organ level clearance mechanisms together may lead to an imbalance in A β homeostasis or in tau spreading mechanisms that help "tip the balance" towards progression of disease.

[H1] Therapeutic implications

Much of the clinical trial landscape for the past 20 years has been dominated by the amyloid cascade hypothesis on the assumption that lowering A β levels will protect neurons. While confidence in the validity of the amyloid cascade remains high, all trials targeting A β to date have failed to improve cognition, and it is now considered possible that anti-A β interventions will need to be carried out at very early stages of the disease, long before symptoms begin, to prevent neural system failure and cognitive impairments. These clinical failures and the emerging evidence of the importance of cellular interactions that impact AD have led to a broadening view of therapeutic targets that may prevent or reverse pathological changes in non-neuronal cell types.

Activation of microglia and astrocytes and the downstream effects on neurons are likely important players early in disease pathogenesis, which offer potential therapeutic targets. Increasing ApoE secretion by astrocytes could be beneficial due to enhancing clearance of A β and potentially enhancing synaptic function¹⁸⁶. Bexarotene, a drug in clinical use to treat skin cancer, has generated interest due to its ability to aid the formation of ApoE lipoprotein particles. A study published in 2012 indicated that bexarotene treatment increased brain ApoE concentrations, reduced A β levels, and reversed cognitive deficits in

APP/PS1 mice¹⁸⁷. This led quickly to clinical trials, however subsequent mouse work has failed to replicate the full benefits seen in the original study¹⁸⁸⁻¹⁹⁰. The initial outcomes from clinical trials have similarly not shown much benefit, only lowering brain A β levels in people who do not have an APOE4 allele and increasing serum triglycerides which could increase cardiovascular risk¹⁹¹. Despite these lukewarm results with bexarotene, there is promise in the idea of harnessing APOE levels as a therapeutic, and it may be that *lowering* ApoE levels will be more helpful as a preventative strategy than raising them. Mouse work indicates that increasing levels of ApoE2, lowering levels of ApoE4, or lowering total ApoE levels in the brain may be beneficial to reduce amyloid levels, enhance synaptic function, and prevent tau mediated toxicity^{38,50,192}.

Another potential therapeutic target based on cellular interactions in AD is inflammation. A large prospective study found that use of non-steroidal anti inflammatory drugs (NSAIDs) is associated with lowered risk of AD¹⁹³. However, thus far, anti-inflammatory drugs have failed in clinical trials for AD despite protecting against many phenotypes in mouse models¹⁹⁴. Human data indicate that anti-inflammatory treatment effects differ at different stages of disease¹⁹⁵, consistent with our conclusion that the role of glia likely changes during the disease course. Although it is still in very early stages, the development of C1q antibodies to treat AD will be of interest based on the involvement of the complement system in synapse loss¹⁹⁶.

At the interface of vascular risk factors and inflammation, diabetes drugs have gained interest as potential therapeutics for AD¹⁹⁷. Promising mouse work indicates that hyperinsulinemia exacerbates AD pathology and that anti-diabetic drugs improve phenotypes in AD models^{198,199}. However, clinical trials with two agonists of the peroxisome proliferator-activated receptor-gamma (PPAR γ), diabetes drugs used to control blood sugar, did not succeed in either symptomatic or prevention trials²⁰⁰ (press release <https://www.takeda.com/newsroom/newsreleases/2018/takeda-tomorrow-trial/>).

Despite disappointing clinical trial results, it is not clear whether therapeutics aimed at APOE, anti-inflammatories, or lowering insulin levels have been adequately studied in humans to date.

Along with therapeutics that target non-neuronal cells, preventative strategies that address the entire NVGU are a promising approach. Recent population based studies indicate that the incidence of dementia in many countries is stable or decreasing²⁰¹. While the reasons for this are not known, it is likely that improved cardiovascular health - through initiatives like reducing smoking - is a contributing factor. Epidemiological studies show an association between exercise and reduced risk of cognitive decline and dementia during ageing^{6,202}. However, to date, interventional trials to prevent or treat dementia by improving cardiovascular health via exercise have inconsistent results trending towards no benefit^{203,204}.

[H1] Concluding Remarks

Healthy brain function relies on intact neural system integrity, which encompasses the intricately choreographed workings of multiple cell types. Here we have outlined that in AD there are changes in multiple cell types and the interactions between them (figure 2). Strong data from genetic and epidemiologic risk factors implicate vascular and glial changes in initiating AD. A disease that plays out over more than a decade, however, might be expected to have multiple moving parts; the role of glia or vasculature abnormalities in each portion of this cascade may change over time, and are also likely to change in the context of aging and other systemic and environmental factors. While it is tempting to try to arrange “cause and effect” arrows in cartoon models of hypotheses, it thus seems very likely that the complexities involved will be difficult to conclusively model in simple systems; we believe that further evaluation of deeply phenotyped human material from patients at different stages of the disease, and with different genetic risk factors and predispositions, will be critical to advance understanding. Nonetheless,

increasing evidence from cell and animal studies implicates the interactions between cell types in damaging synapses and neural circuits, and emphasizes the need for further studies. There is hope that with a clearer understanding of the interactions between cells in the brain and the time course of the changes, we will be able to either prevent or treat AD.

Fig 2

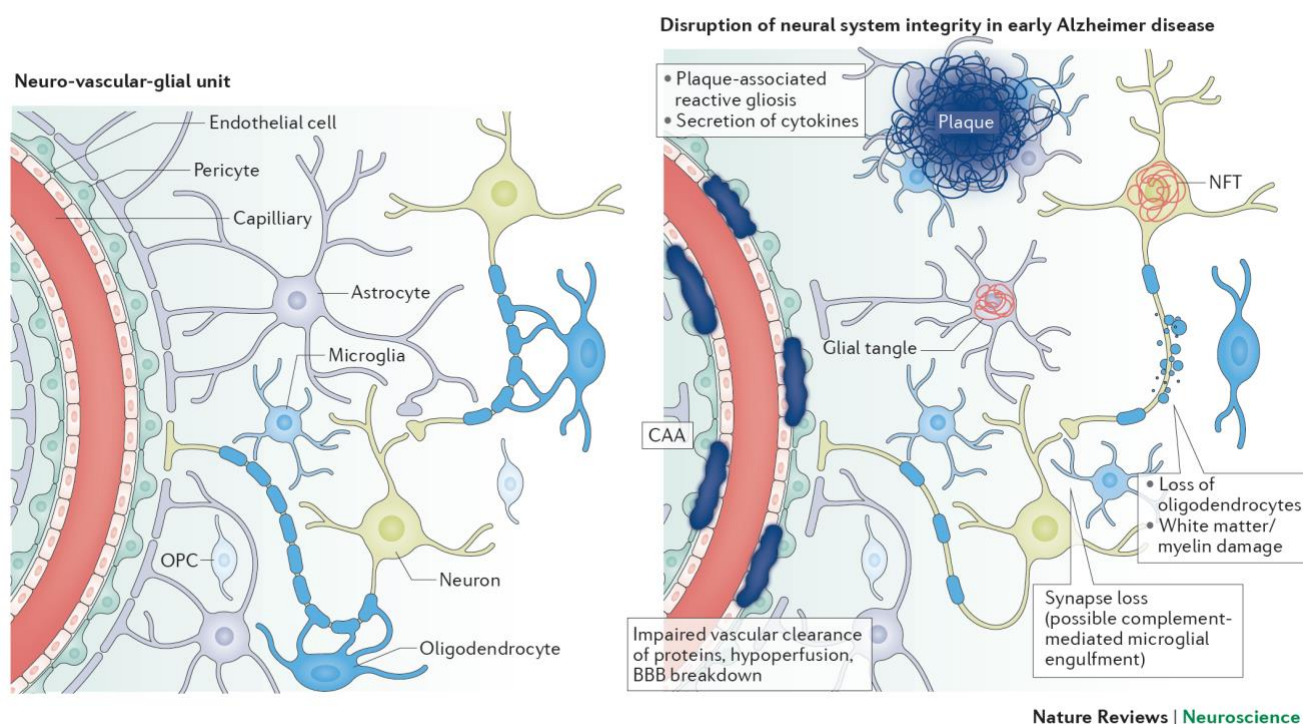


Figure 2: Schematic of the NVU and early changes in Alzheimer's disease. All of the cell types in the brain interact in a complex web to maintain brain function (left). During the early stages of Alzheimer's disease (right), many of these homeostatic processes are impaired, and synapses and neurons are damaged by pathways involving multiple cell types. Vascular integrity (1) is impaired by damage to endothelial cells, astrocytic endfeet, and pericytes and by the build-up of A β along vessel walls in cerebral amyloid angiopathy. These vascular changes cause impairments in vascular clearance of proteins, hypoperfusion, breakdown of the blood brain barrier (BBB). Amyloid plaques (2) are surrounded by a halo of oligomeric A β which damages nearby synapses and neuronal processes. Plaques are also surrounded by astrocytes and microglia which initially serve to clear amyloid protein but after exposure to A β become reactive and secrete cytokines. Tau accumulates in neurofibrillary tangles within neurons (3) which are associated with glial accumulation and neuronal dysfunction and death. Synapses become dysfunctional and disappear (4) in a process that likely involves microglia. White matter integrity is compromised (5) due to loss of oligodendrocytes and damage to myelin caused by chronic hypoperfusion.

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Box 1: Alzheimer's pathology –neurons and beyond

Degenerative changes in the brains of AD patients include the accumulation of "positive" lesions such as amyloid plaques, neurofibrillary tangles, neuropil threads, dystrophic neurites, cerebral amyloid angiopathy, and glial

responses as well as the “negative” lesions of synapse and neuronal loss^{205 206 207}. Plaques are extracellular deposits of the amyloid beta (A β) peptide, which is generated from the sequential cleavage of the amyloid precursor protein by β and γ -secretase enzymes^{208,209}. Plaque deposition begins in the neocortex years before symptom onset, then progressively spreads to hippocampus, diencephalon and striatum, brainstem, and lastly cerebellum²¹⁰. Soluble oligomeric forms of A β rather than plaque fibrils, are toxic to neurons and synapses, but the exact forms of the toxic oligomers and how they confer toxicity remain hotly debated²¹¹.

Neurofibrillary tangles are intracellular lesions composed of misfolded, hyperphosphorylated tau protein^{212,213}. Tangle accumulation occurs early in the transentorhinal cortex then spreads to the entorhinal cortex, hippocampal formation, and later to neocortex^{214,215}. Unlike plaque accumulation, the presence of tangles correlates with neuron loss, synapse loss, and cognitive decline^{216,217}. Like amyloid, it is likely that soluble forms of tau are toxic, but the precise toxic forms remain unknown^{218, 219}.

Drastic brain atrophy occurs in AD particularly in medial temporal lobes and later in frontal lobes²⁰⁵. This progressive atrophy can be observed longitudinally in vivo with structural MRI,²¹⁹ which is increasingly used as a biomarker of disease progression.

Reactive astrocytes (labelled with GFAP in top of figure) and activated microglia (labelled with Iba1, in bottom of figure) accumulate around plaques (arrowheads) and in the vicinity of tangles (arrows)^{45,205} Figure reproduced with permission.

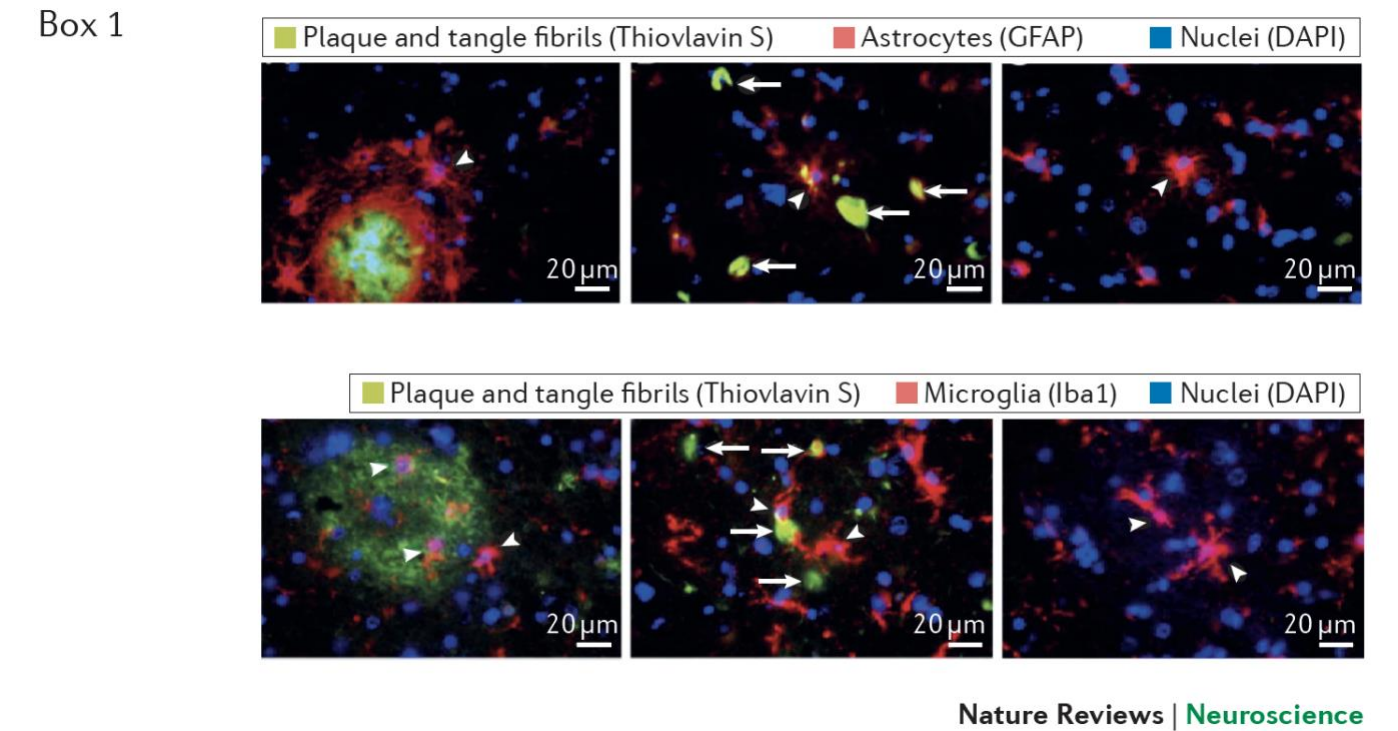


Figure legends

Table 1: Changes in multiple cell types in the brain early in Alzheimer’s disease. This table highlights the normal functions of several brain cell types and how these change in early Alzheimer’s disease pathogenesis.

Cell Type	Normal function	Changes in Early AD	references
microglia	• Trophic support	• Microgliosis	13,220,221

	<ul style="list-style-type: none"> Brain surveillance Phagocytosis of debris 	<ul style="list-style-type: none"> Beneficial and impaired clearance of amyloid and tau Release of neurotoxic and proinflammatory factors Excessive synaptic phagocytosis 	
astrocyte	<ul style="list-style-type: none"> Trophic support Metabolic support Blood-brain-barrier component Synaptic modulation 	<ul style="list-style-type: none"> Astrogliosis Beneficial and impaired clearance of amyloid and tau Release of neurotoxic and proinflammatory factors Synaptic phagocytosis Impaired synaptic homeostasis BBB breakdown 	221-223
oligodendrocyte	<ul style="list-style-type: none"> Trophic support Myelin production 	<ul style="list-style-type: none"> Loss of oligodendrocytes Myelin loss Disrupted neuronal plasticity 	224,225
neuron	<ul style="list-style-type: none"> Electrical and chemical transmission Excitatory and inhibitory synaptic activity Synaptic plasticity, circuit function, cognition 	<ul style="list-style-type: none"> Intracellular tau aggregation Synaptic accumulation of amyloid and/or tau Synaptic dysfunction Synapse loss 	2,97,226
Cerebral Vascular cells <ul style="list-style-type: none"> Endothelial cells Pericytes and smooth muscle cells Perivascular macrophages 	<ul style="list-style-type: none"> Endothelial cells, pericytes, and astrocytic end feet are essential components of BBB Smooth muscle cells control blood flow Perivascular macrophages aid clearance of toxins 	<ul style="list-style-type: none"> Impaired amyloid clearance Cerebral Amyloid Angiopathy BBB breakdown Obstructed blood flow Decreased CNS glucose uptake Oxidative stress 	2,10,162

Table 2

Risk allele	Cell type(s)	gene function(s)	references
<i>APOE4</i>	Astrocytes, microglia	Lipid metabolism	9
<i>ABCA7</i>	All cell types	Lipid metabolism	227,228
<i>CLU</i>	Astrocytes, OPCs	Lipid metabolism, endocytosis	109,227,229
<i>SORL1</i>	Astrocytes	Lipid metabolism, endocytosis	227
<i>TREM2</i>	microglia	Immune response	230-232
<i>PLCG2</i>	Microglia	Immune response	232

<i>ABI3</i>	microglia	Immune response	232
<i>CR1</i>	microglia	Immune response	109,227
<i>CD33</i>	microglia	Immune response	227,228
<i>INPP5D</i>	microglia	Immune response	227
<i>Bin1</i>	Microglia, oligodendrocytes, neurons	endocytosis	227
<i>PICALM</i>	Microglia/endothelial	endocytosis	227,229
<i>ZCWPW1</i>	OPCs, oligodendrocytes, microglia	Epigenetic regulation	227
<i>NME8</i>	All cell types	cytoskeleton	227
<i>EPHA1</i>	oligodendrocytes	Ephrin receptor	227,228
<i>HLA-DRB5-HLA-DRB1</i>	microglia, macrophages	Immune response	227

Table 1: Genetic risk factors for sporadic AD and the brain cell types where they are most highly expressed (for reviews with more exhaustive lists of risk genes, and database where we searched for cell types that express genes see ²³³⁻²³⁵).

Glossary

Amyloid cascade hypothesis: Initially proposed in 1992, this hypothesis posits that the accumulation of amyloid beta (A β) is the initiating factor in Alzheimer's disease pathogenesis, leading to the formation of amyloid plaques, neurofibrillary tangles, neuron loss and clinical dementia.

Innate immune system: Reactive response that utilises chemical mediators to fight infection and clear foreign substances from the body by recruiting specialised immune cells. It can also activate a second wave of adaptive immune response by presenting antigens to adaptive immune cells.

Secretome: All secretable factors released from a cell.

Homeostatic gene: Gene encoding a protein involved in a homeostatic mechanism within the cell

Cytokine: Small releasable signalling proteins that often have immunomodulatory effects. These include chemokines, interleukins and interferons and they can be released by numerous immune cell types, endothelial cells and fibroblasts.

Oligomeric A β : Single molecules of amyloid beta (A β) are known as monomers. These monomers can aggregate to form oligomeric structures of 2 or more monomers, which can then accumulate into larger fibrillar forms of A β and deposit as the hallmark amyloid plaques.

Glymphatic system: Drainage pathway found in the vertebrate central nervous system, which allows cerebrospinal fluid (CSF) to enter the brain alongside penetrating arteries and facilitates the removal of interstitial fluid (ISF) and waste products from the brain.